Evidence that Beetles are Involved in the Rarely-Seen Reproduction of the Chaga Fungus (*Inonotus obliquus*)

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Figure 1. Canker ("chaga") of *I. obliquus* on the side of a living host tree.

Chaga: ubiquitous, enigmatic fungus

Inonotus obliquus is a ubiquitous polyporoid fungus of the Northern Hemisphere and is found in northern latitudes of North America, Europe, and Asia (Bondartsev, 1971). In North America the fungus is a pathogen of birch trees (*Betula* spp.) and very rarely other species including hop hornbeam, *Ostrya virginiana*. The species is very well-known and highly prized commercially for purported pharmacological benefits including cancer-fighting properties found in a cankerous growth (called a "canker," "clinker," or "chaga") that the fungus creates on the main stem or trunk of infected mature host trees (Fig. 1). The health benefits of the fungus have been known among a number of indigenous peoples for centuries (Rogers, 2012) and used by Russian and Scandinavian peoples for more than a century, with a commercially-prepared medicine from chaga being prescribed as a treatment for cancer there for several decades (Pilz, 2012; Spinosa, 2012). More recently, there have been numerous peer-reviewed studies published on the anti-cancer properties (as well as other benefits to human health) of this fungus (Bunyard, 2012; Rogers, 2012; Spinosa, 2012). Internet searches return numerous links to discussion of chaga; few other fungi share the allure and widespread appeal that the chaga fungus currently enjoys. Chaga has been featured in the pages of FUNGI numerous times over the past decade; it's such a hot topic that an entire issue of FUNGI was dedicated to all aspects of this enigmatic fungus (vol. 5, no.3; 2012). "Chaga" is now a household word in mycological circles, as well as among foragers and those involved in the commercial trade of naturopathic, neutraceutical, and medicinal plants/ fungi boom that is currently ongoing. Many shops and online sources offer chaga and preparations of this fungus for sale.

Despite its universal recognizability, many questions about the basic biology of this fungus remain (Bunyard, 2012), including its pathogenicity toward its tree hosts (Blanchette, 1982), its geographic distribution, and sustainability of harvesting this species. Most experts feel wild chaga may be exhausted in a few decades but almost no studies have been published on harvest sustainability (Pilz, 2012). Functionality of the canker, if any, is unknown; a storage sink for nutrients (needed for growth or fruitbody development) have been proposed. Most surprisingly, the life cycle of *I. obliquus* is poorly known (Bunyard, 2012; Millman, 2012) but insects have been suspected to play a key role, and recently Bunyard (2015) provided some evidence that beetles are involved in the sexual reproduction and spore dissemination.

Figure 2. Rarely seen sporocarp of *I. obliquus*. 8, Sporocarp in situ on dead host tree with its discoverer, Sarah Dole.

Chaga: an enigmatic fungus with an enigmatic style of reproduction

The chaga canker is commonly seen in birch forests but the sexual reproductive structure ("basidiocarp" or "sporocarp," Figs. 2–3) is rarely seen (Gilbertson and Ryvarden, 1986; Bondartsev, 1971). Indeed a connection between the two structures wasn't even proposed until as recently as 1938 (Campbell and Davidson, 1938; Millman, 2012). Moreover, a number of factors about the reproductive biology of *I. obliquus* are enigmatic. The basidiocarp is actually little more than a resupinate (crustose) layer of tubes that form on a recently dead host tree. The fungus does not seem to fruit more than one time during the host's lifetime (Gilbertson and Ryvarden, 1986) and most host trees killed by the fungus may never show any sign that the fungus has gone through sexual reproduction. The resupinate layer forms beneath the bark of the host tree which is very unusual for poroid fungi (Millman, 2012). The only other polypore species that forms a fruiting body under bark is *Inonotus andersonii* (Millman, 2012), one of the primary causes of mortality among oaks in southwestern North America

Figure 3. Close up view of sporocarp tubes.

Figure 4.

Figure 5.

Figure 6.

Figure 7.

Figures. 4–7. *Orchesia cultriformis*. 4, Adult (dorsal view). 5, Adult (lateral view). 6, Adult (ventral view). 7, Larva, (lateral view). Photography by Fred Rhoades.

Figure 8. Gut contents of *O. cultriformis* feeding on *I. inonotus* sporocarp tissues. 5, Larva, scale bar = 100 µm. 6, Adult, scale bar = 25 µm. Photography by Fred Rhoades.

(Gilbertson and Ryvarden, 1986).

When the spore-forming resupinate layer forms on host trees it seems to quickly attract mycophagous insects (Gilbertson and Ryvarden, 1986) and primarily beetles (Bunyard, 2015; Bondartsev, 1971). Bondartsev (1971) noted that the fruitbody, when rarely seen, is usually little more than a mass of tunnels and beetle frass and is all but unrecognizable. Based on the attraction by beetles, and the unusual location of fruiting (beneath the host tree's bark), beetles have been suspected to play a role in spore dispersal of this fungus (Millman, 2012).

So it was met with great excitement when one of us (SD) had located

Figures 9–11. The "mother tree," Year Two, looking the worse for wear. Time, beetles, and woodpeckers have taken their toll.

a huge fruiting of this fungus on a recently dead yellow birch tree, *Betula alleghaniensis*, near her home,

Figure 9.

Figure 10.

in Quebec, Canada, approximately 20-25 feet north of the border of Essex County, Vermont (45.012815, -71.611770). Many trips to the "mother tree" were made to observe and collect any insects that arrived and seemed interested in this fruitbody. Many adults and larva of the beetle *Orchesia cultriformis* (Family Melandryidae) were observed feeding on the reproductive tissue of the fungus and collected in jars of alcohol (Figs. 4–7). Beetle IDs were made with the assistance of Margaret K. Thayer, *Curator Emeritus* of the Field Museum of Natural History (Chicago, IL). Three adults and one larva were dissected and photographed under microscopy (by FR), with their gut contents all found to contain hyphae and spores positively identified to be those of *I. obliquus* (Fig. 8–9). Dried pinned adult specimens of *O. cultriformis* are housed, along with sporocarp specimens of *I. obliquus* in the collections of The Field Museum of Natural History (Chicago, IL; in the Insects and Botany collections, respectively), and a final report was published (Bunyard, 2015).

The end of the story … or is it?

The "mother tree" was visited during the second year after the chaga fruiting, and photographed to document its decline. Over time the fruitbody has withered and many holes have been opened up by woodpeckers, no doubt seeking burrowing arthropods (who may have initially be associated with the chaga fruiting) (Figs. 9–11).

Although this was a first-ever account of insect mycophagy (and potential spore dissemination) of the chaga fungus in North America, insect, and especially beetle spore dispersal is known. Overall, arthropods are well-known vectors of spores of many disparate groups of fungi including rusts, smuts, yeasts, and stinkhorns (for a review, see Ingold, 1971). A few studies have focused specifically on polyporoid fungi and insect vectors. Hubbard (1892) may have been the first to note an important connection between beetles and the dissemination of a polypore's spores. Talbot (1952) showed a number of invertebrates that inhabit wood and bark could ingest and then pass viable mycelium and spores (endozoochory) of polypores. More recently, mycophagous Diptera (Bunyard, 2003; Lim, 1977; Tuno, 1999) and mycophagous coleopterans (Nuorteva and Laine, 1972) also were shown to pass viable mycelium and spores of polyporoid fungi. Most recently Lilleskov and Bruns (2005) showed a number of different arthropods could ingest and pass viable spores of a widespread resupinate mycorrhizal fungus (*Tomentella sublilacina*).

Although this current study provides some evidence of zoochory by beetles, to date, no study has determined positively that any animals play a role in spore dispersal for *I. obliquus.* Schigel (2011), in one of the few studies on polypore-associated insects to include *I. obliquus*, found that, in Finland, the larvae and adults of several species of mycophagous and polyphagous beetles are the primary consumers. *Orchesia micans* was noted as being the most commonly reared from *I. obliquus* in their study. Beetles of Family Melandryidae ("false darkling beetles") are commonly found under the bark of mature and rotting trees, and are thought to feed on rotting wood (xylophagous) or fungal hyphae (mycetophagous). Melandryids have been poorly studied (Leschen, 1990; Majka and Pollock, 2006) and their life histories have seldom been described (LeSage, 1991), with most collections coming from adults collected in traps and thus there is no knowledge of larval life histories. Nikitsky and Schigel (2004) also found *O. micans* (along with several other beetle species) to be mycophagous on *I. obliquus* in Russia. In North America, *O. cultriformis* (as well as *Orchesia castanea*) have been associated with *Inonotus hispidus* and *I. dryadeus*; it should be noted that these two species are pileate (form a well-developed soft fleshy basidiome or "bracket" on the host tree), unlike the resupinate *I. obliquus.* Lalibért (1966) originally described *O. cultriformis* from "*Polyporus dryophilus*" (=*Inonotus dryophilus*). Although mycophagous coleopterans have been well-studied in North America, no studies have included the resupinate sporocarp of *I. obliquus.* Nonetheless it seems likely that this species and other resupinate fungi could be dispersed by invertebrates (Lilleskov and Bruns, 2005). Furthermore, zoochory might help explain the widespread distribution of *I. obliquus* and the cryptic nature of its reproduction.

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